Tree Physiology 8, 83–91
© 1991 *Heron Publishing—Victoria, Canada*

91-06811/

Acquired thermotolerance of jack pine, white spruce and black spruce seedlings

R. S. KOPPENAAL,[1] S. J. COLOMBO[1] and E. BLUMWALD[2]

[1] *Ontario Ministry of Natural Resources, Ontario Forest Research Institute, P.O. Box 969, Sault Ste. Marie, Ontario, Canada P6A 5N5*

[2] *Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B3*

Received February 8, 1990

Summary

The acquired thermotolerance of first-year seedlings of jack pine (*Pinus banksiana* Lamb.) hardened at 36, 38, 40 or 42 °C for 90, 180 or 360 minutes and of black spruce (*Picea mariana* (Mill.) B.S.P.) hardened at 34, 36, 38 or 40 °C for 30, 90, 180 or 360 minutes was determined by comparison of needle damage to that of non-hardened seedlings (25 °C) following exposure to temperatures of 49 and 47.5 °C, respectively. Compared to seedlings kept at 25 °C, heat injury sustained from exposure to high temperatures was markedly reduced following hardening for 180 minutes at 36 and 38 °C in jack pine and black spruce, respectively. Increasing the exposure time at 36 °C in jack pine, and at 36 to 40 °C in black spruce, also reduced needle damage. The duration of increased thermotolerance was investigated in jack pine, black spruce and white spruce (*Picea glauca* (Moench) Voss) by comparing heat injury from high temperatures in non-hardened seedlings and in seedlings hardened at 38 °C for 180 minutes a day for either 1, 3 or 6 days. In all three species, the duration of acquired thermotolerance increased with the number of days of heat hardening. For jack pine and white spruce seedlings hardened at 38 °C for 6 days, increased thermotolerance persisted for at least 14 and 10 days, respectively, after the end of the hardening treatment. In contrast, the thermotolerance of black spruce seedlings hardened at 38 °C for 6 days remained elevated for only 4 days.

Introduction

High surface temperatures frequently occur on boreal planting sites during summer and have been directly implicated in coniferous seedling mortality and plantation failure (United States Department of Agriculture 1937, Issac 1938, Smith 1951). Tree seedlings grown in containers under standard greenhouse conditions are particularly susceptible to injury from high temperatures when they are actively growing (Koppenaal and Colombo 1988). For these reasons, factors affecting the thermotolerance of tree seedlings are of practical significance.

Exposure to high, sublethal temperatures is known to increase thermotolerance in agronomic species (Alexandrov 1956, Lomigan 1961, Yarwood 1961). The acquired thermotolerance is due, at least in part, to the synthesis of thermostable heat shock proteins (Key et al. 1981, Lin et al. 1984). Seasonal variation in thermotolerance has been reported in coniferous species (Lange 1967, Reidmuller-Schölm 1974, Bannister and Smith 1983), but little else is known about the capacity of tree seedlings to acquire thermotolerance (Gauslaa 1984). The intent of this study was to determine the capacity for, and duration of, acquired thermotolerance in first-year jack pine

(*Pinus banksiana* Lamb.), white spruce (*Picea glauca* (Moench) Voss) and black spruce (*Picea mariana* (Mill) B.S.P.) seedlings following hardening at supraoptimal temperatures.

Materials and methods

Experiments were conducted independently with jack pine, white spruce and black spruce seedlings. Seed of the three species was obtained from general northeastern Ontario collections (site region 3E (Hills 1960)). Seedlings were grown in a 2/1 peat/vermiculite mixture in flats of Japanese paperpot FH408 containers (70 cm^3) in a greenhouse according to cultural practices similar to those described by Carlson (1983). One week before heat hardening, seedlings were transferred with paperpot containers to smaller plastic trays of 25 to 30 seedlings with a 2.5 cm peat buffer around the perimeter of the tray and placed in a growth chamber at 25 °C day and 18 °C night temperatures in an 18-h photoperiod.

Hardening treatments were applied in growth chambers to jack pine (16 weeks old) and black spruce (24 weeks old) to determine the effects of temperature and exposure time on thermotolerance. Jack pine seedlings were hardened at 36, 38, 40 or 42 °C for 90, 180 or 360 minutes. Black spruce seedlings were hardened at 34, 36, 38 or 40 °C for 30, 90, 180 or 360 minutes. One tray of 30 seedlings was allotted to each hardening treatment. A tray of non-hardened seedlings of each species was kept at 25 °C. Within a species, all hardening treatments were scheduled to finish concurrently. One day following heat hardening, the terminal 5 cm (jack pine) or 7 cm (black spruce) of the stem was clipped from 10 seedlings from each treatment and placed in sealed plastic bags. The bags were submerged in a circulating water bath for 30 minutes at 47.5 °C for black spruce and 49 °C for jack pine. In preliminary tests, these temperatures were determined to be the approximate threshold for heat injury to actively growing shoots of these species. To ensure that damage was not caused by factors other than exposure to high temperatures, an additional group of shoot tips from the non-hardened seedlings of each species were placed in a bag but were not exposed to the water bath. Shoot tips from all treatments were then placed in test tubes, two shoots per tube, with their cut ends submerged in a small volume of water. Test tubes were left in a growth chamber (25 °C day, 18 °C night) for 10 days following the high temperature treatment, after which shoot tips were classified according to percent foliar damage estimated visually in 10-percent intervals.

Further experiments were conducted to determine the duration of acquired thermotolerance following heat hardening in jack pine (14 weeks old), black spruce (24 weeks old) and white spruce (23 weeks old) seedlings. These experiments were conducted as previously described, except that seedlings were all hardened at 38 °C for 180 minutes a day for either 1, 3 or 6 consecutive days, the single or final treatment occurring on the same day for a given species. Non-hardened seedlings were kept at 25 °C day and 18 °C night over the same period. Heat-hardened seedlings were also kept at these temperatures before and after each 180-minute hardening treatment. Two trays containing 40 seedlings each were allocated to each

hardening treatment (25 °C, 38 °C × 1, 3 or 6 days). Trays were watered daily to prevent desiccation. Excised shoot tips were subsequently exposed to water bath temperatures of 47.5 (black spruce), 48 (white spruce) or 49 °C (jack pine) for 30 minutes, starting one day after hardening treatments were completed and continuing up to 10 (white spruce), and 14 (jack pine) days after hardening treatments ended, or until increased thermotolerance was no longer observed, as in black spruce. Damage in shoot tips was assessed as described above.

Nonparametric tests were used to analyze the data because of a lack of homogeneity of variance among treatments. The median percent damage value of each shoot tip was ranked according to the Kruskal-Wallis test and differences in heat injury were compared among temperatures and among exposure times by one-way analysis of variance of the ranked data followed by nonparametric multiple comparison tests (Zar 1984). In experiments to examine the duration of acquired heat tolerance, the same procedure was used to compare differences in needle damage among treatments (25 °C, 38 °C for 1, 3 or 6 days) at a given number of days following heat hardening.

Results and discussion

Thermotolerance of black spruce seedlings, as reflected in the amount of needle damage, generally developed in a temperature- and time-dependent manner (Figure 1). Needles of non-hardened black spruce seedlings or those hardened at 34 °C

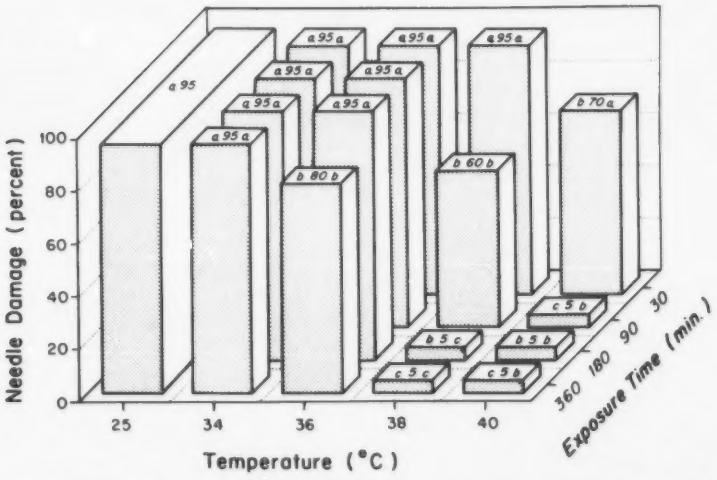

Figure 1. Median percent needle damage of black spruce seedlings exposed to 47.5 °C following treatment at 25 °C (non-hardened) and supraoptimal temperatures (heat hardened) for a range of exposure times. Exposure time was not measured in seedlings at 25 °C. Bar values preceded by the same letter indicate no significant ($P < 0.05$) differences among temperatures at the same exposure time, as determined by the Kruskal-Wallis test for analysis of variance of ranked data. Bar values followed by the same letter indicate no significant ($P < 0.05$) differences among exposure times at like temperatures.

were 95 percent damaged at 47.5 °C. Injury to black spruce caused by exposure to 47.5 °C was markedly less in seedlings previously hardened either at 38 °C for at least 180 minutes, or at 40 °C for 90 minutes or more.

In jack pine, injury sustained by exposure to 49 °C decreased with increasing exposure time at 36 °C, with little needle damage occurring in seedlings hardened for 180 or 360 minutes (Figure 2). Jack pine seedlings hardened at 38, 40 or 42 °C also acquired greater thermotolerance compared to non-hardened (25 °C) seedlings, which showed extensive needle damage after exposure to high temperatures.

The high temperature treatments used in this investigation (47.5–49 °C), which were injurious to non-hardened seedlings, were within the range of high temperatures found lethal to several other tree species (Baker 1929, Lorenz 1939, Seidel 1986). Our results confirm the findings of earlier studies with crop plants, in which tolerance to lethal high temperatures increased following exposure to supraoptimal temperatures (Alexandrov 1956, Lomigan 1961, Yarwood 1961). As in the present study, an increase in hardening temperature or exposure time has been reported to result in enhanced tolerance to otherwise lethal high temperatures (Feldman et al. 1967, Alexandrov et al. 1977).

Gauslaa (1984) investigated heat resistance in needles of dormant *Pseudotsuga taxifolia* (Poir.) Britton and *Tsuga heterophylla* (Raf.) Sarg. hardened at 24 to 38.5 °C for up to 46 hours. Although thermotolerance increased with hardening temperature, acquisition of thermotolerance was reported following exposure to temperatures as low as 24 °C for as little as 5 hours. Unfortunately, it is unclear what temperature

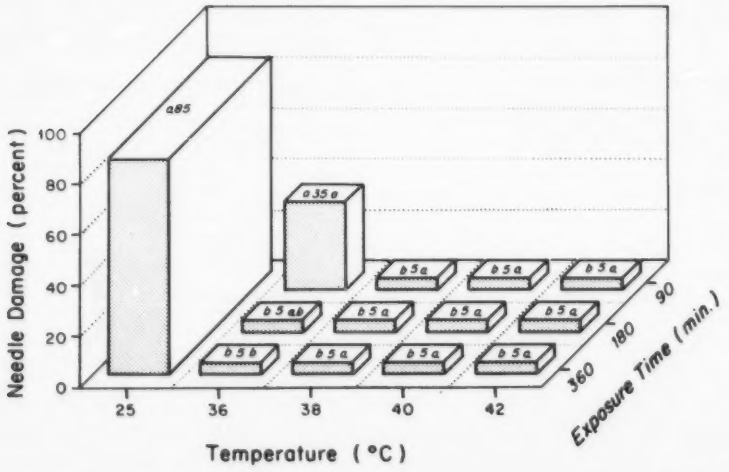

Figure 2. Median percent needle damage of jack pine seedlings exposed to 49 °C following treatment at 25 °C (non-hardened) and supraoptimal temperatures (heat hardened) for a range of exposure times. Exposure time was not measured in seedlings at 25 °C. Bar values preceded by the same letter indicate no significant ($P < 0.05$) differences among temperatures at the same exposure time, as determined by the Kruskal-Wallis test for analysis of variance of ranked data. Bar values followed by the same letter indicate no significant ($P < 0.05$) differences among exposure times at like temperatures.

these conifers were exposed to before hardening (twigs were apparently collected directly from the field in early November). Reports of acquired thermotolerance at normal growing temperatures (Kislyuk and Vaskovsky 1981, Gauslaa 1984) contrast with the results of our study and others (Alexandrov 1977, Key et al. 1985), although this disagreement may be explained in part by the variation among studies in ambient temperatures. In the present study thermotolerance was not acquired in black spruce at temperatures below 36 °C following a single exposure for 360 minutes or less. Although no lower temperature limit for acquired thermotolerance was determined in jack pine, it is unlikely to be much lower than in black spruce because jack pine seedlings hardened at 36 °C for 90 minutes were not significantly more thermotolerant than non-hardened seedlings. However, in a study with potted black spruce seedlings, increased thermotolerance was induced by several days of repeated exposure to temperatures as low as 32 °C (Koppenaal, unpublished observations).

The duration of acquired thermotolerance in jack pine, white spruce and black spruce seedlings increased with the number of days of hardening at 38 °C for 180 minutes per day (Figure 3–5). Seven days after hardening treatments ended, jack pine seedlings hardened for 1, 3 or 6 days at 38 °C were more thermotolerant at 49 °C than non-hardened seedlings (Figure 3). Increased thermotolerance persisted after 14 days in seedlings hardened for 6 days at 38 °C, but in seedlings hardened for 1 and 3 days at 38 °C damage sustained at 49 °C was not significantly lower than in non-hardened seedlings. Although the median percent needle damage of non-hardened (25 °C) jack pine in this experiment (Figure 3) was 35 to 50 percent lower than

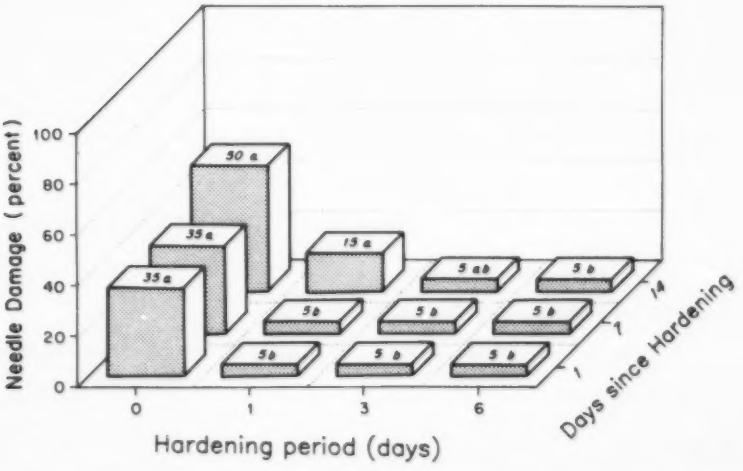

Figure 3. Effect of the number of days of heat hardening (38 °C × 180 min day^{-1}) on median percent needle damage of jack pine seedlings exposed to 47.5 °C 1, 7 and 14 days after heat hardening. Bar values followed by the same letter indicate no significant ($P < 0.05$) differences among hardening treatments (0, 1, 3 and 6 days) at the same number of days following heat hardening as determined by the Kruskal-Wallis test for analysis of variance of ranked data.

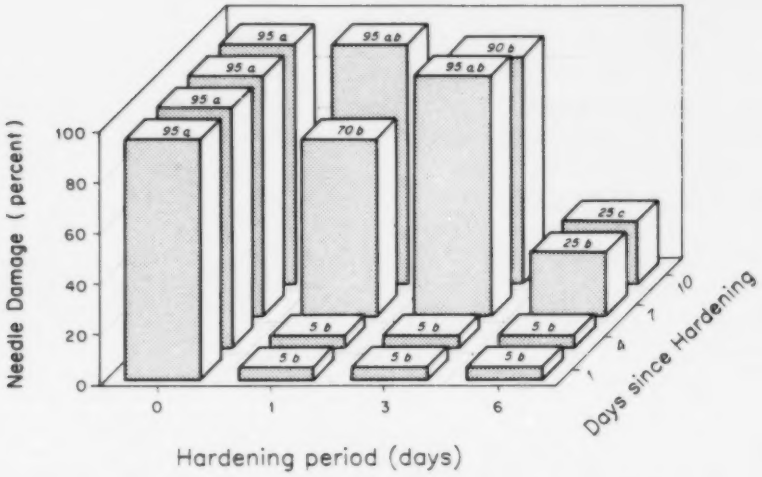

Figure 4. Effect of the number of days of heat hardening (38 °C × 180 min day^{-1}) on median percent needle damage of white spruce seedlings exposed to 48 °C 1, 4, 7 or 10 days after heat hardening. Bar values followed by the same letter indicate no significant ($P < 0.05$) differences among hardening treatments (0, 1, 3 and 6 days) at the same number of days following heat hardening as determined by the Kruskal-Wallis test for analysis of variance of ranked data.

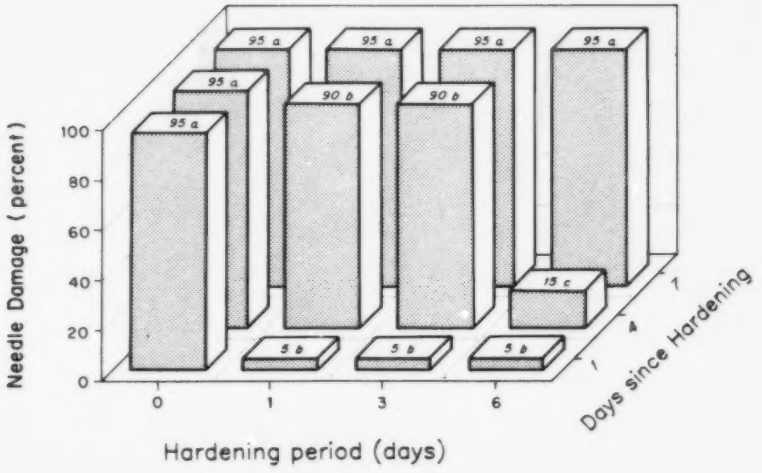

Figure 5. Effect of the number of days of heat hardening (38 °C × 180 min day^{-1}) on median percent needle damage of black spruce seedlings exposed to 49 °C 1, 4 or 7 days after heat hardening. Bar values followed by the same letter indicate no significant ($P < 0.05$) differences among hardening treatments (0, 1, 3 and 6 days) at the same number of days following heat hardening as determined by the Kruskal-Wallis test for analysis of variance of ranked data.

in the experiment presented in Figure 2, the difference in the means of the damage values between the non-hardened seedlings of the two experiments was not more than 19 percent. It is likely that differences in the amount of heat injury in non-hardened jack pine is attributable to uncontrolled factors between the two experiments, possibly in relation to diurnal shifts in thermotolerance (Colombo, unpublished observations).

Shoots of hardened (38 °C for 1, 3 or 6 days) white spruce seedlings showed little or no damage when exposed to 48 °C one and four days later, whereas shoots of non-hardened seedlings were 95 percent damaged (Figure 4). Ten days after heat hardening, increased thermotolerance was still evident in white spruce seedlings hardened at 38 °C for 6 days, although heat injury in seedlings hardened for shorter periods was similar to that in non-hardened plants.

Acquired thermotolerance in black spruce seedlings lasted for a shorter period than in jack pine or white spruce seedlings (Figure 5). One day after hardening treatments ended, thermotolerance was greater in all heat-hardened black spruce seedlings compared to non-hardened seedlings. Four days after heat hardening, only black spruce seedlings hardened for the longest period (6 days) showed much less heat injury compared to non-hardened seedlings, which were 95% damaged by exposure to 47.5 °C. Black spruce shoots of all treatments were 95% damaged 7 days after hardening treatments ended. The duration of acquired thermotolerance in jack pine and white spruce is several days longer than that reported for agronomic species, which do not differ in thermotolerance from non-hardened plants within 2 to 7 days after supraoptimal heating (Schroeder et al. 1963, Alexandrov 1977, Drozdov et al. 1984). Although the rate of de-hardening is influenced by ambient temperatures (Alexandrov 1977), the ambient temperature in the present study (25 °C) was within the range of ambient temperatures reported in other investigations. In a treatise by Alexandrov (1977), plant response to repeated supraoptimal heating is discussed with reference to Shukhtina (1964) who reported that subsequent exposure to the same temperature did not affect thermostability, provided that an optimum dose of the first heat treatment was properly chosen. However, our results clearly demonstrate that repeated exposure to supraoptimal temperatures sustains increased thermotolerance for a longer period of time than a single exposure, which is consistent with other studies (Yarwood 1967, Drozdov 1984), although in the latter studies, temperatures were either increasing or decreasing during the treatment period.

In summary, the results of this study indicate that tree species attain increased thermotolerance in response to supraoptimal temperatures, a phenomenon well documented for agronomic species for which it has been shown that thermotolerance develops in a time- and temperature-dependent manner (Alexandrov 1977, Key et al. 1985). Moreover, the effect is cumulative because the duration of acquired thermotolerance in the three species studied was extended by repeated exposure to high temperatures.

The exposure of plants to high temperatures causes an alteration in gene expression, with the induction of heat shock protein synthesis and the repression of some

normally expressed proteins (Sachs and Ho 1986). It is believed that heat shock proteins play a role in the acquisition of thermotolerance in higher plants, although a well defined role for any plant heat shock protein has not yet been elucidated. *In vitro* evidence indicates that tree species also respond to heat treatment with the rapid synthesis of heat shock proteins (Colclough, M, Koppenaal, R, Colombo, S.J. and Blumwald, E., in preparation). The characterization of heat shock proteins and their role in acquired thermotolerance in conifers will be reported in a subsequent paper.

Acknowledgments

We acknowledge the diligent technical assistance provided by Stewart Blake. We also thank Jo-Ann Tonge and Donna-Marie McKay for typing the manuscript. This work was funded by the Canada-Ontario Forest Resources Development Agreement (Project No. 33500).

References

Alexandrov, V.Ya. 1956. Cytophysiological analysis of thermoresistance of plant cells and some problems of cytoecology. Bot. Zh. 41:939–961.

Alexandrov, V.Ya. 1977. Cells, molecules and temperature. Ecol. Studies 21. Springer-Verlag, Berlin.

Baker, F.S. 1929. Effect of excessively high temperatures on coniferous reproduction. J. For. 27:949–975.

Bannister, P. and P.J.M. Smith. 1983. The heat resistance of some New Zealand plants. Flora 173:399–414.

Carlson, L.W. 1983. Guidelines for rearing containerized conifer seedlings in the prairie provinces. Can. For. Serv. Inf. Rep. NOR-X-214E.

Drozdov, S.N., A.F. Titov, V.V. Talanova, S.P. Kritenko, E.G. Sherudilo and T.V. Akimova. 1984. The effect of temperature on cold and heat resistance in growing plants. I. Chilling sensitive species. J. Exp. Bot. 25:1595–1602.

Feldman, N.L., V.Ya. Alexandrov, I.G. Zavadskaya, I.M. Kislyuk, A.G. Lomigan, M.I. Lutova and A. Jaskuliev. 1967. Heat hardening of plant cells under natural and experimental conditions. *In* The Cell and Environmental Temperature. Ed. A.S. Troskin. Pergamon Press, Oxford, pp 152–160.

Gauslaa, Y. 1984. Heat resistance and energy budget in different Scandinavian plants. Holarc. Ecol. 7:1–78.

Hills, G.A. 1960. Regional site research. For. Chron. 36:401–423.

Issac, L.A. 1938. Factors affecting establishment of Douglas-fir seedlings. U.S. Dept. Agric. Circ. No. 486, 45 p.

Key, J.L., C.Y. Lin and Y. Chen. 1981. Heat shock proteins in higher plants. Proc. Natl. Acad. Sci., USA 78:3526–3530.

Key, J.L., J. Kimpel, E. Vierling, C.L. Lin, R.T. Nagao, E. Czarnecka and F. Schoffl. 1985. Physiological and molecular analyses of heat shock response in plants. *In* Changes in Eukaryotic-Gene Expression in Response to Environmental Stress. Eds. B.G. Atkinson and D.B. Walden. pp 327–348.

Kislyuk, I.M. and M.D. Vaskovsky. 1981. The reaction of arctic plants to the temperature increase under experimental conditions. Bot. Zh. 66:1710–1720.

Koppenaal, R.S. and S.J. Colombo. 1988. Heat tolerance of actively growing, bud-initiated, and dormant black spruce seedlings. Can. J. For. Res. 18:1103–1105.

Lange, O.L. 1967. Investigations on the variability of heat-resistance in plants. *In* The Cell and Environmental Temperatures. Ed. A.S. Troshin. Pergamon Press, Oxford, pp 131–141.

Lin, C.Y., J.K. Roberts and J.L. Key. 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their subcellular localization. Plant Physiol. 74:152–160.

Lomigan, A.G. 1961. Changes in the resistance of plant cells after a short action of a high temperature. Tsitologiya 3:430–439.

Lorenz, R.W. 1939. High temperature tolerance of forest trees. Agric. Exp. Stn. Tech. Bull. 141, Univ. Minnesota.

Riedmuller-Schölm, H.E. 1974. The temperature resistance of Alaskan plants from the continental boreal zone. Flora 163:230–250.

Sachs, M.M. and T.H. Ho. 1986. Alteration of gene expression during environmental stress in plants. Ann. Rev. Plant Physiol. 37:363–376.

Schroeder, C.A. 1963. Induced temperature tolerance of plant tissue *in vitro*. Nature 200:1301–1302.

Seidel, K.W. 1986. Tolerance of seedlings of ponderosa pine, Douglas-fir, Grand fir and Engelmann spruce for high temperatures. Northwest Sci. 60:1–7.

Shukhtina, H.G. 1964. The influence of repeated heat-hardening on thermostability of plant cells. *In* Tsitologicheskiye osnovy prisposableniya rastenii k faktoram sredy (collection of works (Sbornik) Cytological Aspect of Adaptation of Plants to the Environmental Factors). Moscow, Leningrad, Izd. Nauka, pp 26–30.

Smith, D.M. 1951. The influence of seedbed conditions on the regeneration of eastern white pine. Conn. Agric. Exp. Stn. Bull. No. 545, New Haven, 61 p.

United States Department of Agriculture. 1937. Heat more injurious than lack of moisture during drought. Lake States For. Exp. Stn. Tech. Note No. 125, St. Paul, MN.

Yarwood, C.E. 1961. Acquired tolerance of leaves to heat. Science 134:941–942.

Yarwood, C.E. 1967. Adaptation of plants and plant pathogens to heat. *In* Molecular Mechanisms of Temperature Adaptation. Ed. C.L. Prosser. Washington: Amer. Assn. Advan. Sci. pp 75–89.

Zar, J.H. 1984. Biostatistical analysis. 2nd Edn. Prentice-Hall, Inc., N.J.